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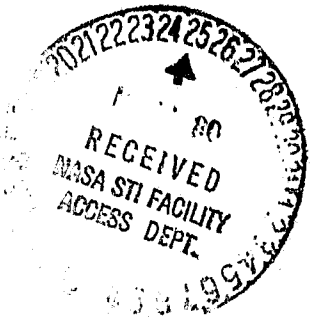

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STATE OF THE MINERAL COMPONENT OF RAT BONE TISSUE DURING HYPOKINESIA AND THE RECOVERY PERIOD

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Long term mobility restriction in animals induces changes in the metabolism of /30* calcium, phosphorus and protein in mineralized tissues [1, 2, 5]. However until the present time no comprehensive study had been made in respect to the condition of the mineral component of bone tissue under conditions of hypokinesia and during the recovery period. The present investigation is devoted to this problem.

Method. The study was done on 135 mongrel albino rats weighing initially 140-150 g. Background data were obtained from 10 intact rats. Hypokinesia was produced by putting the animals in narrow cages for 20, 40, 60 and 100 hours. At the end of each hypokinetic period part of the animals were put on a free exercise program (recovery period).

After rats had been killed their femurs were removed and subjected to rentgenography in the absence of an intensification screen on a TUR-60 apparatus using Mikrat-200 film, shooting conditions: 10 mA, 25 kv, 4 sec. Viewing the sagittal and frontal projections on the rentgenograms through an MBS-2 microscope equipped with an ocular micrometer the central portion of the bone was measured for diaphysis and osteomedullar canal as well as overall thickness of the cortical layer from opposite sides. In the degreased and dehydrated femoral bone (entire right and the left in the distal epiphysis) the following indices were determined: bone density (g/cm^3), ash content per unit volume (mineral saturation g/cm^3) and in percent of dry /31 weight (ash content in percent). Bone volume was determined by weighing on torsion scales in the air and distilled water. The bones were ashed in a muffle furnace at 700°C for 7 hours and the ash weighed. The formula used in calculations was:

*Numbers in the margin indicate pagination in the foreign text.

$$V = \frac{P_{\text{в воздухе}} - P_{\text{в воде}}}{d}; \quad \text{b' зольность} = \frac{P_{\text{золы}}}{P_{\text{сухой}}} \times 100;$$

$$\text{f плотность} = \frac{P_{\text{сухой}}}{V}, \quad \text{g минеральная насыщенность} = \frac{P_{\text{золы}}}{V},$$

Key: V. Bone volume.
P. Bone weight.
a. In air.
b. In water.
d. Specific gravity of water.
b' Ash content.
c. Ash.
e. Dry.
f. Density.
g. Mineral saturation.

From the central portion of the diaphysis of the left femur ring-shaped fragments 3 mm across were sawed and measured for the same indices as the whole bone. For greater accuracy in water measurements surface tension on the bone was diminished by the addition of a small amount of detergent. Contact quantitative microrentgenography was used for differential quantitative determination of microstructural mineralization [3, 6]. This method was used to determine optical density of microrentgenograms for transverse sections of the central portion of the femoral diaphysis as compared with the density of a picture of a standard graded tapered sample. Photometry of the microrentgenograms was done in the subperiosteal, intermediary and subendosteal zones of the section and the results were expressed in grams of the mineral component per cubic centimeter. A study was also made of the effect of hypokinesia on the intensity of Ca^{45} incorporation in the proximal epiphysis and diaphysis of the femur. For this purpose 24 hours before being sacrificed the animals received Ca^{45} intraperitoneally in the form of a chlorite diluted in an isotonic NaCl solution at a dosage level of 2 microcuries per animal. The accepted method [4] was used for treating the material and determining radiochemically the Ca^{45} in the ash. Data were expressed in percent of the isotope dose administered.

Results and Evaluation. Under conditions of hypokinesia the weight of the rats and the weight and volume of the femur were lower than the corresponding figures for the control (Fig. 1). Hypokinesia for 20, 40 and 60 days led to a reduction in the thickness of the cortical layer of the diaphysis in a front-back direction by 6-11% and after 100 days by 25.9% ($P<0.05$) as compared with the control. Much less marked was the reduction of the cortical layer looked at from the lateral surfaces of the femur on day 20 and especially on days 40 and 60 of the experiment (18.4% and 22.6% respectively compared with the control, which is about twice larger than the parameters of

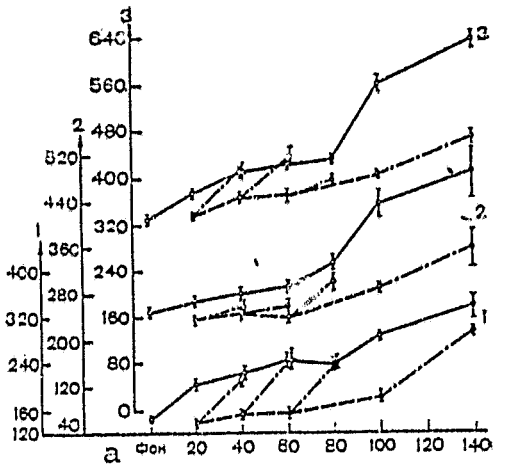

Fig. 1. Change in body mass of rats (1, in g) and of the mass (2, in mg) and volume (3, in mm^3) of femoral bone in hypokinesia and during recovery period. Here and in Fig. 2 and 3: solid line - control, broken line - hypokinesia, dot-dash - recovery period, X axis - time (days), a -background

measurement in the front-back direction; Fig. 2). Density, ash content and mineral saturation of the overall femur and epiphysis separately diminished in the experimental animals, yet there was no evidence of a direct link between the manifestness of these changes and the length of the experiment. Density and mineralization of the diaphysis bone tissue increased with the length of hypokinesia. For example, by the 100th day of the experiment the density, ash content and mineral saturation of the cortical layer of the diaphysis had increased by 5.9 , 5 and 15% respectively ($P < 0.05-0.01$), i. e. mobility restriction was not resulting in the development of osteoporosis. Decrease in the mineral content of the entire bone was linked with attenuation of the cortical layer while the width of the osteomedullar channel was maintained. The drop in epiphysis mineralization seems to be connected /32 with attenuation of the osseous trabeculae of the porous material.

The figure for mineral saturation, obtained by the volume-weight method, reflects the content of phosphoro-calcium salts in a unit volume of a fragment of the cortical layer and is a direct function of the degree of mineralization of the microstructures of the osseous tissue and conversely of the total volume of the vascular channels and resorption cavities. The effect of these "nonmineralized" areas on the amount of mineral saturation was eliminated by the microrentgenological study. As a result it was established (Fig. 3), that minerals were unequally distributed in different layers of the femoral cortical layer: the intermediary was the most mineralized and the subperiosteal the least.

Under hypokinetic conditions mineral saturation of osseous tissue increases in all zones of the femoral diaphysis, the dynamics of this indicator being the same as for control animals but occurring at a higher level. There was a more marked increase of mineral saturation for the strata of the subperiosteal layer than the intermediary.

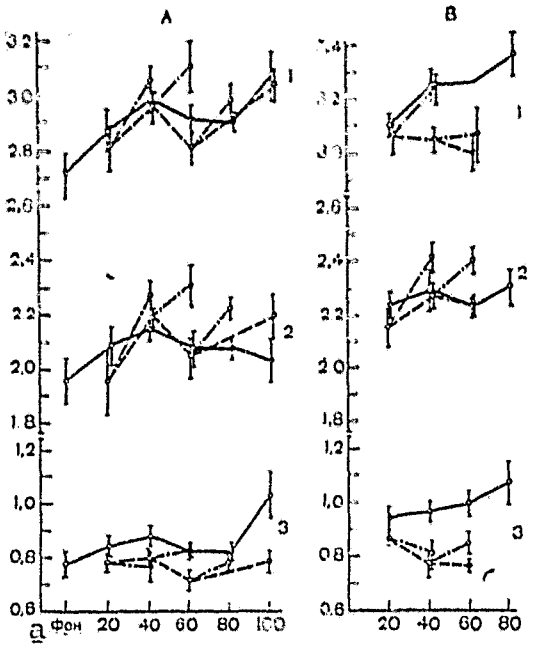

Fig. 2. Changes in cross-sections of rat femoral bone in hypokinesia and during recovery. A - front-back, B - side, X axis - time (days), Y axis - width of diaphysis (1), medullar channel (2), cortical layer (3) in mm.

The data obtained may be interpreted as severe inhibition of bone tissue formation in hypokinesia corresponding to severe attenutation of the osseous cortical layer.

By day 20 of the recovery period following 20, 40 and 60 days of hypokinesia the animals rapidly took on weight and their weight became perfectly normal; following 100 days restricted mobility the animals' weight did not reach the control level (see Fig. 1). Femoral volume during the recovery period normalized only with 20 or 40 day hypokinesia. Bone mass was not restored in a single period of the study because of the marked loss of density. In the recovery /33 period the rats' overall femoral density went down particularly in the epiphysis. Density parameters and those for mineral saturation of osseous tissue in the cortical layer of the diaphysis increased to a larger extent, than under hypokinesia alone. In contrast to these findings results of the microrentgenographic study following 60 and 100 days of hypokinesia showed normalization of mineral saturation for microstructures in all investigated zones of the diaphysis (see Fig. 3). The thickness of the cortical layer tended to normalize when measured front-back but the lateral measurement remained low (see Fig. 2). The width of the osseomedullar channel of the rat femur during recovery following hypokinesia of varying lengths increased 7-11% when compared with the control ($P<0.05$) thus explaining its density. The radioisotope study showed, that for control rats the intensity of Ca^{45} incorporation in the femoral diaphysis was in inverse ratio to growth. 20 days of restricted mobility caused the level of such incorporation in the diaphysis and epiphysis to go down (85.8 and 77.8% in respect to the control). On day 40 of hypokinesia the incorporation of the isotope in these tissues increased slightly but on day 60 went down once more on an average of 10% compared to the control. During the recovery period following 20 and 40 days of hypokinesia the Ca^{45} content inthe epiphysis and diaphysis of the femur tended to reduction; after 60 days of hypokinesia it rose insignificantly in the diaphysis and substantially in the epiphysis (21.5% compared to

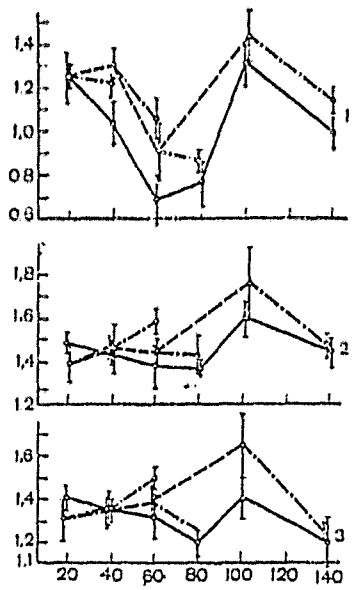

Fig. 3. Changes in mineral saturation of microstructures in cortical layer of rat femoral bone in hypokinesia and in recovery. Zones: 1 - subperiosteal, 2 - intermediary, 3 -subendosteal. X axis - time (days), Y axis - mineral saturation in g/cm^3

control; $P < 0.05$).

Thus, long term restricted mobility for growing rats slows down increase in mass and femoral volume, reduces the density of porous matter in the epiphyses and retards development of the diaphysis' cortical layer. At the same time there is an increase in the density of this same layer due to an increase in mineral saturation of osseous tissue microstructures. Mineral saturation increases most in the subperiosteal layer, evidence of retardation in the oppositional growth of the bone and consequently of the inhibition of young bone formation. Ca^{45} incorporation into bone tissue under conditions of hypokinesia tends to reduction, probably a reflection of the repression of the osteogenetic process. Normalization of the bone's mineral component during the recovery period following various lengths of hypokinesia is partial in character.

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